A Model of Flagellar and Ciliary Functioning Which Uses the Forces Transverse to the Axoneme as the Regulator of Dynein Activation

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Ciliary and flagellar motion is driven by the dynein-tubulin interaction between adjacent doublets of the axoneme, and the resulting sliding displacements are converted into axonemal bends that are propagated. When the axoneme is bent in the normal beating plane, force develops across the axoneme in the plane of the bend. This transverse force (t-force) has maximal effect on the interdoublet spacing of outer doublets 2-4 on one side of the axoneme and doublets 7-9 on the opposite side. Episodes of sliding originate as the t-force brings these doublets into closer proximity (allowing dynein bridges to form) and are terminated when these doublets are separated from each other by the t-force. A second factor, the adhesive force of the dynein-tubulin attachments (bridges), also acts to pull neighboring doublets closer together. This force resists termination of a sliding episode once initiated, and acts locally to give the population of dynein bridges a type of excitability. In other words, as bridges form, the probability of nearby bridges attaching is increased by a positive feedback exerted through the interdoublet spacing. A conceptual working hypothesis explaining the behavior of cilia and flagella is proposed based on the above concepts. Additionally, the feasibility of this proposed mechanism is demonstrated using a computer simulation. The simulation uses a Monte Carlo-type algorithm for dynein attachment and adhesive force, together with a geometric evaluation of the t-force on the key microtubule pairs. This model successfully develops spontaneous oscillations from any starting configuration (including a straight position). It is compatible with the physical dimensions, mechanical properties and bridge forces measured in real cilia and flagella. In operation, it exhibits many of the observed actions of cilia and flagella, most notably wave propagation and the ability to produce both cilia-like and flagella-like waveforms.

Key words: dynein arms, nexin links, radial spokes, relaxation oscillator, doublet microtubules, biological oscillators, computer model

INTRODUCTION

The flagellar (and ciliary) axoneme is a highly conserved structure common to eukaryotes in both the plant and animal kingdoms. It is widely accepted that the motor protein dynein, which forms the "arms" of the outer doublet microtubules, is an ATPase which generates force between the outer doublets and results in a sliding displacement between adjacent doublet microtubules [Summers and Gibbons, 1971, 1973]. It is also generally

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I dedicate this work to the memory of the late Dr. Robert Rikmenspoel, mentor, colleague and friend. His scientific contributions have played a significant role in the development of this work.
accepted that each doublet slides baseward on its higher numbered neighbor [Sale and Satir, 1977]. Therefore, given the circular arrangement of the outer doublets, the forces produced by the doublet pairs on opposite sides of the axoneme act to bend the flagellum in opposite directions.

While much has been elucidated about the basic force-generating mechanism, the question still remains: how does the axoneme function to produce orderly episodes of dynein-tubulin sliding leading to coordinated beating? Many of the facts that are germane to this question have long been in evidence in the literature. Several investigators have noted that the spacing between the outer doublets in fixed preparations is too large to permit dynein arms to bridge the distance from the A microtubule of one doublet to an adjacent B microtubule [Warner, 1978; Gibbons and Gibbons, 1973]. However, it has also been shown that during rigor, when dynein bridges form, the doublets are pulled together and the diameter of the axoneme is thereby reduced [Gibbons and Gibbons, 1973; Warner, 1978; Warner and Mitchell, 1978; Zanetti et al., 1979]. As early as 1975, Summers suggested that the presence of the nexin links between the outer doublets dictates that sliding must be accompanied by stretching of the nexin, which in turn would tend to pull the adjacent doublets closer [Summers, 1975]. Warner [1978] has observed that flagella treated with Mg$^{2+}$ maintain a bent configuration. In these flagella the axoneme is compressed in the plane of bending and takes on an oval appearance in cross section.

It has also been demonstrated that bending a flagellum or flagellar fragment which has lost coordination can restore beating and activate bend propagation [Lindemann and Rikmenspoel, 1972]. In a splayed axonemal preparation, Kamiya and Okagaki [1986] showed that sliding episodes between adjacent doublets will spontaneously terminate when bending and shear reach a certain critical limit. Therefore, the available evidence suggests that both sliding initiation and termination can be self-regulated by shear developing between doublets.

Evidence from three different types of organisms also suggests that there are structural constraints that govern the functional organization of the axoneme. Studies on a marine ciliate, Beröe [Tamm and Tamm, 1984], sea urchin spermatozoa [Sale, 1986] and both rat and bull sperm [Lindemann et al., 1992; Kanous et al., 1993] all point to a common conclusion, that the axoneme is divided by a natural partition formed from doublet elements 3 and 8 linked to the central pair. This arrangement was first suggested by Afzelius [1959]. It has also long been known that doublets 5 and 6 are permanently linked in many (if not all) organisms [Afzelius, 1959; Gibbons, 1961]. These structures prohibit, or at least greatly limit, sliding displacement in one plane of the axoneme, and are probably responsible for the semiplanar nature of the flagellar beat in most organisms [Gibbons, 1961; Brokaw, 1965, 1972; Rikmenspoel, 1965; Phillips, 1972].

In a recent report [Lindemann, 1994], the key information on the functional organization of the axoneme was combined with a method for treating the transverse forces (t-forces) generated in a bending axoneme. The conceptual scheme that emerged used the t-force developed across the axoneme as the single determinant for initiating or terminating episodes of dynein-tubulin interaction. This hypothesis was dubbed the “Geometric Clutch” mechanism of the axoneme. When this scheme was incorporated into a computer simulation of a flagellum, it demonstrated both the ability to establish stable oscillations, and the ability to develop and propagate waves of bending similar to that of a living flagellum.

In this report, the same conceptual design is further developed and refined. The entire simulation is scaled to centimeters, grams and seconds (cgs units) to test its feasibility under the natural constraints of realistic physical scale. A physiological number of force-producing bridges is employed, and rather than using a simple on-off switching algorithm, the probability of their attachment/detachment is influenced by the t-force. Finally, the effects of adhesive forces contributed by currently attached bridges are also considered in the bridge switching algorithm. The resulting computer simulation exhibits behaviors remarkably like those of living cilia and flagella.

**ANALYSIS AND MODEL**

The flagellar and ciliary beat is predominantly restricted to the direction perpendicular to the central pair of the axoneme [Tamm and Horridge, 1970]. Two special features explain the restricted beat plane. Firstly, there is solid evidence for a stable central partition, a linking of the central pair with doublets 3 and 8 [Tamm and Tamm, 1984; Sale, 1986; Lindemann et al., 1992]. Secondly, the 5 and 6 doublets are permanently bridged and do not slide relative to each other [Afzelius, 1959; Gibbons, 1961; Warner and Satir, 1974; Lindemann and Gibbons, 1975; Olson and Linck, 1977; Linck, 1979; Lindemann et al., 1992].

Due to the circular arrangement of the outer doublets of the axoneme, and the polarity of interdoublet sliding [Sale and Satir 1977], the dynein arms on opposite sides of the axoneme act to bend the axoneme in opposite directions (doublets 2–3 and 3–4 act together in one bend direction, while 7–8 and 8–9 act in the opposite as indicated in Fig. 1). With the exception of doublets 5 and 6, which are often permanently bridged, some interdoublet sliding may occur between each of the
adjacent doublet pairs. However, due to the structural considerations mentioned above, and the resulting planar beat, most interdoublet sliding will occur between elements 2–4 on one side of the axoneme, and between elements 7–9 on the opposite side (as shown in brackets). In the computer simulation of a ciliary/flagellum, two pairs of forces (force couples), corresponding to the force generated between elements 2 and 4 on one side and elements 7 and 9 on the opposite side, have been used to approximate the force-producing apparatus of the axoneme, as shown schematically. Since the two force couples act to bend the flagellum in opposite directions, they have been designated as the P (principal) and R (reverse) dynein bridge sets. The micrograph of a ciliary axoneme was provided by Dr. Robert Hard, University of Buffalo Medical School.

In modeling the axoneme, the analysis has been simplified by focusing on two shear force couples, one representing the relative force developed between elements 2 and 4, and the other between elements 7 and 9. Bending torque is then the product of the shear force times the effective lever arm, where the effective lever arm is the center to center distance between elements 2 and 4 or between 7 and 9 (the effective diameter, D). This simplification is illustrated in Figure 1 and is employed in modelling the flagellum. It divides the force-producing dynein bridges into two files, called the P (principal) and R (reverse) bridges.

**Computed Simulation of an Axoneme**

The computer programs used to stimulate a flagellum/cilium were written in Microsoft QuickBasic. The approach taken for this study was to take an initial data set of thirty angles, specified in radians, and construct a segmented "flagellum." Each of the thirty angles determines the placement of the next segment, starting from the first segment which is placed at the coordinate origin (0,0). From the initial segment angles and positions, values are calculated for the change in angle per unit of segment length (curvature, \( \frac{d\theta}{ds} \)), and the change in angle from the initial segment angle (shear angle) and each is stored in a data file. The simulated flagellum is then recalculated over a specified number of iterations, where decay of the curvature values is determined by a fractional multiplier. In that way, each segment joint decays exponentially towards 0 curvature in the passive model.

Superimposed on this very basic simulation of an elastic rod are the perturbations originating from sliding and active bridge cycling, as well as viscous drag. Moments of torque, derived from stretching passive interdoublet links and from active dynein bridges, are used to determine an instantaneous equilibrium position of the structure. The active bridge forces and passive stretching forces from both sides of the axoneme are added together into a total force file, as they would be added (or subtracted) as longitudinal forces onto the doublet microtubules interacting with the central partition. The total longitudinal force at segment \( n \) (\( F(n) \)), multiplied by the doublet spacing dimension (effective diameter, D), yields the total torque acting at \( n \). This is then balanced by finding the curvature, \( \frac{d\theta}{ds} \), at which the local stiffness \( I_0E \) \( \times \frac{d\theta}{ds} \) is equal and opposite to the total instantaneous torque:

\[
\frac{d\theta}{ds} \times I_0E = F(n) \times D \quad (1a)
\]

where:

\[
F(n) = 2 \times \sum_{i=n}^{30} F_L(i) + \sum_{i=n}^{30} F_P(i) + \sum_{i=n}^{30} F_R(i) \quad (1b)
\]

\( F_L \) is the longitudinal component of force derived from stretching the passive elastic links between the doublets on each side of the axoneme. \( F_P \) and \( F_R \) are forces contributed by the activated bridges of each modeling segment in the principal \( F_P \) and reverse \( F_R \) bend directions. The curvature solution to Equation 1a at each segment \( n \) \( (\frac{d\theta}{ds}) \) then defines the equilibrium curvature of that particular segment joint. The passive model is then modified to decay exponentially to this equilibrium curvature, instead of to 0 curvature. Treating the forces generated by the passive linkages and active bridges ad-
ditive, in the same data file, is similar to the theoretical treatment devised by Brokaw [1971, 1972].

To simulate the effect of viscous drag on the flagellum, the following algorithm was employed. At each iteration, at each segment, the previous and current positions of the flagellum are compared to calculate segment velocities (V), and the moment of drag (M_{DRAG}) at n is the sum of moments of drag on each of the more distal segments:

\[ M_{DRAG}(n) = \sum_{i=n}^{30} \text{drag coefficient} \times V(i) \times \Delta L_{n}(i) \times \Delta s \]

(2)

where \( L_{n} \) is the lever arm (distance from the current segment) and \( \Delta s \) is the segment length. The decay constant at each segment is adjusted at each iteration to make the torque due to drag at that segment stay roughly equal to the sum of the elastic and active torques, which is the physical mandate:

\[ M_{ACTIVE} + M_{ELASTIC} - M_{DRAG} = 0 \]

(3)
as recognized earlier by Machin [1963] and Rikmenspoel [1971].

To simulate flagella-like motion, where the base segment is free to pivot, the boundary condition that viscous drag torque = 0 at the base segment was employed. To accomplish this, the drag was recalculated with a feedback correction of the base angle derived from the magnitude of the previous drag calculation. The process was repeated until the drag torque at the base was reduced below an acceptable cutoff. This method provided a very stable base correction algorithm, using either a base completely free to rotate, completely fixed, or any variation between these two extremes by choosing the appropriate cutoff value.

**Modeling the Dynein Switching Mechanism**

The key hypothesis constituting the core of the model proposed here is that dynein heads form force-producing bridges to subtubule B with a probability proportionate to the interdoublet spacing. In turn, force between the doublets in the direction transverse to the flagellar axis and in the plane of the flagellar beat (t-force) is responsible for regulating the interdoublet spacing. Therefore, the t-force is the primary determinant of dynein activation and deactivation, and is consequently responsible for initiating and terminating episodes of microtubule sliding.

Several working assumptions have been made to permit a simple simulation of dynein bridge switching:

1. The dynein is in its activated state.
2. The sole determinant of dynein head binding to tubulin is physical proximity of the dynein head to the B tubule. Due to variations in molecular kinetic energy, this shows up as a probability function since the greater the doublet spacing, the fewer dynein heads will possess sufficient kinetic excursion to contact and bind the neighboring B tubule.
3. When a dynein head binds to the B tubule of the adjacent doublet, a force is exerted between the doublets. This force has a component longitudinal to the axis of the doublets, which drives interdoublet sliding, and a component transverse to the axis of the doublets, which contributes a positive adhesive force drawing the doublets closer.
4. For the purpose of modeling, a constant value has been assigned to the sliding force contributed per active dynein head.

Some of these assumptions are oversimplifications and serve only as a first approximation to facilitate modeling. It is anticipated that refinements will be incorporated as the model is upgraded in the future.

**T-Force**

The method used to treat the t-force was developed in a prior report [Lindemann, 1994]. As shown in Figure 2, when the flagellum bends, tension or compression develops on each of the doublets by the active pulling of dynein bridges (F_A) and by passive stretching of interdoublet links (F_L). At any calculation segment (n) in the computer simulation, the total longitudinal force on a doublet will be:

\[ F_{TOTAL}(n) = \sum_{i=n}^{30} F_L(i) + \sum_{i=n}^{30} F_A(i) \]

(4)

which is the sum of the active and passive components of force contributed by more distal segments, n through 30. Only the forces distal to each point are totaled to calculate the longitudinal force. This is justified by the principle that for a linear element under tension (or compression) Newton’s First Law dictates that for every force (action) there is an equal and opposite force (reaction). Therefore, the tension (or compression) on the linear doublet, generated from forces distal to each point, is always in balance with an equal and opposite force contributed by the structural and active elements proximal to that same point. The magnitude of both forces can consequently be determined by integrating the total force in one direction. Using the distal force provides a uniform method (applicable at every position) that has been successfully employed in other ciliary and flagellar models, and serves to make a complex system tractable.

The total longitudinal force, times the curvature
Fig. 2. Forces distorting the axoneme. A schematic drawing of an axoneme during an episode of dynein activity is shown. As the dynein arms generate force between the adjacent doublets on the activated side of the axoneme (shown in A), doublets 5–7 are pushed baseward and doublets 9 and 1 are pushed tipward. The fixed basal attachment at the basal body forces the structure into a curve as sliding occurs. The cumulative tension, shown by the arrows parallel to the axoneme, causes an outward transverse force (t-force) on elements 9 and 1. This force is largest near the base. Similarly, the cumulative compression on elements 5–7 causes an outward t-force on those elements. This outwardly directed t-force couple (labeled 't') will ultimately terminate the dynein bridge engagement. A section of the opposite side of the same axoneme is represented in B. On this (passive) side, the dynein bridges are not engaged, but sliding is also occurring (as indicated by the arrows). The force causing the sliding comes from the opposite side and must be sufficient to stretch the passive interdoublet linkages between these doublets. This transferred force has also been included in modeling the behavior of the axoneme.

change per restraining element, yields the t-force resulting from the longitudinal strain on the doublets. Since this contribution comes from the cumulative action of many active and passive elements along the axoneme, it is referred to as the global component of t-force, and is expressed as:

$$\text{Global t-force} = \frac{d\theta}{ds} \times F_{\text{TOTAL}} \quad (5)$$

When the passive linkages between doublets are stretched, they also directly contribute a vectorial force component transverse to the axoneme. The magnitude of this force is dependent on the angle of the links to the doublets ($\phi$). This direct, local contribution to t-force is always positive (squeezing the doublets together), and its magnitude can be expressed as:

$$\text{Local t-force} = F_T = F_E \sin \phi \quad (6)$$

where $F_E$ is the whole elastic force magnitude.

The longitudinal component is found in similar fashion:

$$\text{Longitudinal component} = F_L = F_E \cos \phi \quad (7)$$

The sign of this component is determined by the direction of interdoublet sliding. This component is used in the calculation of the total longitudinal force, $F$ (Equation 1b) which drives the movement of the simulation, and is used to determine Global t-force (Equation 4). At each segment, $\phi$ can be found from the interdoublet sliding at segment $n$. The stretch of the passive linkers ($D_p$) can be found by Pythagorean Theorem [see Lindemann, 1994]. If the passive links are assigned an elastic constant ($K_E$) then:

$$F_E = K_E \times D_p \quad (8)$$

The value of $K_E$ is a fundamental unknown that must be assigned a value in the model.

The total t-force at each segment is a combination of both the global and local t-forces:

$$\text{Total t-force} = \text{Local t-force} + \text{Global t-force} \quad (9)$$

Each side of the axoneme experiences a different total t-force because the active dynein bridge contributions are different on the doublets of the two sides. When the axoneme bends, there must be some sharing of force from bridges on one side in order to passively bend the opposite side. This transferred force is schematically displayed in Figure 2B. It is transferred via the interdoublet connections, and is responsible for stretching the interdoublet links on the passive side as the entire flagellum is bent. While this transfer of active force is probably a small fraction of the total active force, it cannot be zero. To take this transfer force into account in the formulation of the t-force, the final expression for the t-force at segment $n$ on the two sides of the axoneme each contain a fractional carryover (transfer factor) from the opposite side:

$$t \text{-force}_p(n) = F_T(n) + \left[ \frac{d\theta}{ds} \times \sum_{i=n}^{30} \left[ F_L(i) + F_R(i) + (\text{transfer factor} \times F_E(i)) \right] \right] \quad (10a)$$

$$t \text{-force}_R(n) = F_T(n) + \left[ \frac{d\theta}{ds} \times \sum_{i=n}^{30} \left[ F_L(i) + F_R(i) + (\text{transfer factor} \times F_E(i)) \right] \right] \quad (10b)$$
where $F_p$ and $F_r$ are the active forces in the principal and reverse files. $F_T$ is the local t-force and $F_L$ is the longitudinal passive elastic component (from Equations 6 and 7, respectively).

**Bridge Adhesion**

When dynein-tubulin bridges form, they must also produce an adhesive force between adjacent doublets. Since the interdoublet spacing in a nonrigor axoneme is greater than the resting length of the dynein arms, each newly attached dynein should contribute a positive adhesive force acting to decrease the interdoublet separation. The force derives from the elastic stretching of the dynein stalk when a bridge is formed. In the extreme case of a flagellum in rigor, the interdoublet separation should be noticeably decreased, and this has been verified experimentally [Gibbons and Gibbons, 1973; Warner, 1978; Warner and Mitchell, 1978; Zanetti et al., 1979].

Figure 3 graphically depicts the adhesion force concept. As bridges attach, they pull adjacent doublets slightly closer, increasing the chance that neighboring dynein heads will also attach. Conversely, detachment of dynein bridge increases the probability of further detachment by decreasing the total adhesion holding doublets together. To incorporate this behavior into the simulated flagellum, the magnitude of the active bridge force per segment is used to alter the probability of bridge attachment/detachment.

**Dynein Bridge Switching Algorithm**

At each iteration of the computer simulation, a series of random numbers is generated for each modeling segment. Each number generated corresponds to one of the dynein heads found on the key doublets on the two sides of the axoneme (see Fig. 1). This equates to $= 4 \times 10^6$ dynein heads/cm times the segment length. The two populations of bridges are designated as the P and R bridge files. Each random number has a value between 0 and 1. Its value is compared to the base (resting) probability threshold to determine if bridge attachment occurs. P and R file bridges can be assigned different resting probability thresholds.

The base or resting probability (Base.P) is modified by the magnitude of the t-force to yield an active probability (Active.P) at each new iteration. Where:

$$\text{Active.P} = \text{Base.P} + (\text{t-force} \times \text{scaling constant}) \quad (11)$$

The scaling constant makes t-force magnitude compatible with the probability range, and positive t-forces make the probability of attachment increase.

At each iteration, the effects of bridge adhesion must also be accounted for. After the active probability is calculated from the t-force at each segment, the program enters a conditional loop and evaluates the effect of bridge attachment at each segment. The adhesive contribution (adhesion) to the bridge switching probability is found using the following formula:

$$\text{Adhesion} = \text{Active bridge force} \times \text{scaling constant} \times (1 - \text{probability of attachment}) \quad (12)$$

where the scaling constant adjusts the magnitude of adhesion to make it compatible with a 0 to 1 probability range, and the probability of attachment is the latest active probability. Using this formula, as more bridges attach and the probability of attachment nears 1, the contribution of additional bridge attachments declines progressively. This is consistent with the concept that as attached bridges pull the doublets closer, subsequent
bridge attachment will have less of an effect on doublet spacing.

Just as for the t-forces, the adhesive forces will be affected by transaxonemal force transfer (see Fig. 3B). A fractional portion of the positive adhesive force on one side of the axoneme will act to pull doublets apart on the opposite side. Therefore, a final correction is made in the adhesion values for the P and R bridge sets before adjusting the switching probabilities:

\[
\text{Adhesion}_{P} = \text{Adhesion}_{P} - (\text{transfer factor} \times \text{Adhesion}_{R}) \\
\text{Adhesion}_{R} = \text{Adhesion}_{R} - (\text{transfer factor} \times \text{Adhesion}_{P})
\]

(13a) \( (13b) \)

Since this side-to-side force transfer is mediated through the same structures as in the global t-force calculation, the same transfer factor is employed in both calculations.

In the switching algorithm employed in the simulation, the global t-force and active probability are calculated from the current shape and bridge distribution. The program then enters a loop and evaluates the effect on bridge attachments at each segment. The redistribution of bridges changes the adhesive contribution to the dynamic probability (Dynamic.P):

\[
\text{Dynamic.P} = \text{Active.P} + \text{Adhesion}[n + 0.5(n-1) + 0.5(n+1)]
\]

(14)

After finding the dynamic probability threshold for both the P and R bridge files, the loop reevaluates the bridge attachments with the dynamic probabilities used as the switch points. The process repeats until the loop detects less than a 10% change in the total dynein bridge attachment. In other words, the effect of adhesive force on bridge attachment must be at least 90% completed before the loop is exited. The distribution of bridge attachment on exiting the loop is then used as the source for the calculation of longitudinal force between doublet elements in the P and R files. Total active force is found by adding the contribution from all distal segments \( n \) to 30 at each segment \( n \).

RESULTS

The computer simulation, using t-forces and adhesion forces to regulate the probability of dynein attachment, displays many characteristics of living cilia and flagella. As long as the resting probabilities of P and R bridge attachment are greater than 0, the simulation will initiate motility from any reasonable starting position, including a straight position (see Fig. 4). The simulation is capable of stable oscillations that can repeat indefinitely. When the numbers used in the simulation are scaled to cgs units, it is possible to maintain the stable oscillatory behavior using dimensions, forces and physical characteristics compatible with real cilia and flagella (Table I). If the length specified in the simulation is cilia-like (10 \( \mu \text{m} \)), and the resting probability of P-bend dynein bridge attachment is several times greater than the resting probability of R-bend dynein bridge attachment, the resultant beat is very cilia-like. Figure 5B shows the beat of a simulated 10 \( \mu \text{m} \) cilium with the base-level bridge attachment probabilities set at 0.05 for the P bridges and 0.01 for the R bridges. The beat has two distinct phases, one resembling an effective stroke and the other resembling a recovery stroke. The bridge attachment pattern during the cycle (Fig. 5B) clearly displays the cause of the asymmetry. A residual patch of bridges near the distal end of the flagellum fails to deactivate, persisting from the effective stroke well into the recovery stroke (Fig. 5C). In the recovery stroke phase, the P wave propagates along the simulated cilium. This arises spontaneously from the self-organizing nature of the system. Timing constants, imposed propagation and forcing functions have not been used in any aspect of the simulation.

If a longer, flagellar-length structure is simulated, with a base that is allowed to pivot, the behavior of the simulation becomes much more flagellum-like (Figs. 5A). Waves of bending form at the base and propagate down the length of the structure. Bend propagation occurs in both the P and R bend directions. Figure 5A also shows that the episodes of dynein bridge activation also propagate along the flagellum.

The t-forces responsible for bridge attachment and detachment in the cilium are shown in Figure 6. The upper plot shows the t-force acting on the P bridges and the lower plot displays the R bridge t-force. The t-force takes on an orderly organization and form derived from the self-organization of the simulation. T-force magnitudes are largest near the base, where the integral of bridge forces and passive forces is also largest. It has a propagating component, but can also exhibit pronounced episodes of positive (activating) activity distributed over most of the ciliary length (demonstrated in Plot 1 in the upper graph of Fig. 6). The presence of such episodes can explain the almost simultaneous activation of most of the P bridges. This behavior is needed to produce an effective stroke.

The simulation was found to oscillate over a fairly wide range of values for transaxonemal force transfer (from 0.0 to 0.5). However, values in the range of 0.3 to 0.1 consistently produced the best facsimiles of cilia-like and flagella-like beating. The simulations displayed in Figure 5 used transfer factor values of 0.16 for the flagellum and 0.2 for the cilium.
TABLE I. Modeling Parameters for Ciliary/Flagellar Simulation

<table>
<thead>
<tr>
<th></th>
<th>Flagellum</th>
<th>Cilium</th>
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<tbody>
<tr>
<td>Length (cm)</td>
<td>0.003</td>
<td>0.001</td>
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<tr>
<td>No. of segments</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Segment length (ΔS) (cm)</td>
<td>1.0 × 10⁻⁴</td>
<td>3.3 × 10⁻⁵</td>
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<tr>
<td>Functional diameter (D^f) (cm)</td>
<td>1.0 × 10⁻⁵</td>
<td>1.0 × 10⁻⁵</td>
</tr>
<tr>
<td>Iteration interval (sec)</td>
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<td>0.0001</td>
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<tr>
<td>Drag coefficient (dyne cm⁻²sec)</td>
<td>0.028</td>
<td>0.028</td>
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<tr>
<td>Passive stiffness (K_p) (dyne cm⁻²)</td>
<td>2.0 × 10⁻¹³</td>
<td>2.0 × 10⁻¹³</td>
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<tr>
<td>Force per active dynein head (dyne)</td>
<td>4.2 × 10⁻⁸</td>
<td>1.2 × 10⁻⁷</td>
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<tr>
<td>Elastic constant per nexin link (K_E) (dyne/cm)</td>
<td>0.03</td>
<td>0.06</td>
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<tr>
<td>Transfer coefficient P - R</td>
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<tr>
<td>Resting probability of dynein bridge attachment (Base P)</td>
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<tr>
<td></td>
<td>0.04 (for reverse bend bridges)</td>
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<tr>
<td>t-force scaling factor^c</td>
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<td>5,000</td>
</tr>
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<td>Adhesion scaling factor^d</td>
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<td>32,000</td>
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<tr>
<td>Dynein heads per segment</td>
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<td>In principal file</td>
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<td>130</td>
</tr>
<tr>
<td>In reverse file</td>
<td>400</td>
<td>130</td>
</tr>
</tbody>
</table>

^aFunctional diameter is based on the spacing between doublets 2–4, and between doublets 7–9.
^bBased on a 100 nm nexin spacing.
^cFrom t-force to bridge attachment probability.
^dFrom adhesion force to bridge attachment probability.

Fig. 4. Start-up and arrest patterns of simulated cilia and flagella. The simulated start-up behavior of a cilium-like 10 μm axoneme with a fixed base (A) and a flagellum-like 30 μm axoneme with a base free to pivot (B) each was started from a straight initial position. Every fourth iteration is displayed for the short axoneme corresponding to 0.0004 ms intervals, and every sixth iteration is shown for the longer axoneme (0.0006 ms intervals). Modeling parameters are as given in Table I. The adhesion force generated by random bridge attachments causes the initial excitability that starts an episode of interdoublet sliding and initiates movement. If switching is arrested by greatly reducing the feedback from t-force (reducing the t-force scaling factor to 100), both the cilium-length axoneme and the flagellum-length axoneme show an arrest configuration (C and D, respectively). The arrest position is the result of a balance between active torque from the dynein bridges and passive torque from stretched interdoublet links.

The ratio of bridge force to the value of K_E was also found to be important. Values of K_E which produced life-like arrest positions when the t-force switching was turned off (see Fig. 4C and D) also produced life-like beating. This may provide a reasonable criteria for the choice of a value for K_E.

It should be noted that both the flagellar simulation and the ciliary simulation are produced using exactly the same switching algorithm. Furthermore, minor changes in the resting probability of bridge attachment in the P and R bend directions can produce a diverse array of beat patterns, many of which are observed in nature.

DISCUSSION

In the hypothetical construct presented here, the structure and geometry of the axoneme contribute to the creation of force transverse to the axis of the axoneme (t-force), and this force is the principal regulator of the dynein-tubulin interaction. The behavior that results from this self-organizing system can be classified as a form of relaxation oscillator. The element of hysteresis, a necessary part of relaxation oscillators, is provided by the dynein bridges that contribute an adhesive force. This force maintains each episode of active sliding until the t-force is strong enough to overcome the adhesive contribution. Figure 7 illustrates the principal events in the beat cycle. The force of dynein bridge adhesion provides a type of excitability wherein attachment of a few bridges (possessing sufficient kinetic energy to make the initial attachment) locally decreases the interdoublet spacing, thereby increasing the probability of additional attach-
As seen in Figure 6, t-forces are largest at the base of the cilium/flagellum, and it is in the region near the base that t-force initiates bridge deactivation. Once oscillation begins, the t-force distribution becomes the organizing principle governing the onset and termination of each subsequent dynein-tubulin sliding episode. Events in a complete cycle of oscillation are schematically represented in Figure 7. Each new episode of bridge attachment is initiated by a positive t-force and a resultant cascade of attachment. The attached bridges act to reverse the bend direction. Once the bend direction reverses, the same bridges ultimately contribute the force to generate a large negative t-force near the base of the structure. This large negative t-force, seen in Figure 6 during operation of the model, overpowers the bridge adhesion. Once bridge adhesion is overcome, a cascade of detachment propagates along the cilium (or flagellum) with the aid of a negative t-force travelling wave (Figure 6).

As developed here, the computer simulation operates well incorporating physical values consistent with published figures based on experimental determinations. The values of the passive stiffness and the drag coefficient were taken from Rikmenspoel and Rudd [1973]. The dynein bridge force used in the ciliary model as shown in Figure 5 was $1.2 \times 10^{-7}$ dynes per dynein head. The value used in the flagellar simulation was $4.2 \times 10^{-8}$ dynes/dynein head. Published measured values for dynein force range from $\approx 5.0 \times 10^{-8}$ dynes/head [Kamimura and Takahashi, 1981; Gittes et al., 1993] up

![Ciliary t-Force Profiles](image)

Fig. 6. The t-force profiles of the cilium simulation. The values of the t-force function at intervals during the beat cycle of the cilium simulation are plotted versus position along the cilium. A shows the t-force for the P bridge side of the axoneme, and B displays the t-force for the R bridge side. The numbered plots are at 26 iteration intervals (2.6 ms), and are distributed over one beat cycle. Note the organized propagation of the t-force, especially the negative bridge-terminating episodes. Also note that the basal initiation and termination of new episodes of bridge activity can be explained by the fact that t-force is maximal near the base of the axoneme.

Fig. 7. The beat cycle of the axoneme. In this simplified schematic, the events on the P and R sides of the axoneme are illustrated to present, in a step-wise format, the hypothetical mechanism by which the axoneme develops oscillations. 1: Starting in the straight position, both bridge sets have a base level of random bridge attachments. 2: A cascade of attachments on one side (usually the side with the higher base level probability of attachment) begins an episode of sliding and, due to force transfer, simultaneously inhibits the opposing side (see Fig. 3B). 3: As bending increases, a negative t-force develops on the active side of the axoneme and is strongest near the base. Force transfer continues to inhibit the opposite side, but becomes weaker as detachment of dynein bridges proceeds. 4: A propagating area of bridge detachment on the P bridge side is accompanied by a positive t-force from passive bending on the R bridge side, and this ensures a cascade of bridge activation on the R side. 5: Curvature is now reversing due to the R bridge forces. P bridges are temporarily inhibited by force transfer from the R bridge side. 6: The curvature is now favorable for production of a negative t-force near the base on the R bridge side, and deactivation begins there. Meanwhile, a new cycle is beginning on the P bridge side, as positive t-force from passive forces originating from stretching the passive interdoublet links and residual active bridges contribute to activation. Arrows labeled P are passive force contributions originating from stretching the passive interdoublet links. Arrows labeled A are active forces from bridges, and A° indicates active force transferred from the opposite side.
Fig 5. Simulated flagellum and cilium. The output of the axonemal simulation is displayed for a full beat cycle. In A, a 30 µm long "flagellum" with a freely pivoting base is displayed every 12th iteration. In B, a 10 µm long "cilium" with an anchored base is shown every 8th iteration. First and last iterations are numbered, and the direction of movement of the cilium is indicated with an arrow. The modeling parameters are given in Table 1, and are scaled to the physical dimensions of an axoneme in cgs units. The pattern of dynein bridge attachment along the axoneme is also displayed below each simulation. The iterations shown are the same as for the plotted simulations. At each iteration, thirty entries are displayed for the P bridges (upper) and the R bridges (lower). They are presented in order from the base to the tip. The entry number (from 0 to 9) indicates the percentage of engaged bridges (i.e., 7 = 70–79%, 8 = 80–89%, etc.). The episodes of bridge engagement, propagation, and detachment can be followed through the beat cycle.
P–Bridges

1. Random bridge attachment

2. Cascade of bridge attachment due to adhesion

3. Initiation of detachment

4. Propagation of detachment

5. Delayed initiation due to force transfer

6. New episode of attachment

R–Bridges

1. Random bridge attachment

2. Inhibition due to force transfer from P side

3. Delayed attachment due to force transfer

4. Initiation of attachment

5. Propagation of attachment

6. Initiation of detachment

Fig. 7.
dynein force values requires information not yet in evidence, such as the number of bridges engaged at any instant in time, the duration of force production by a single active bridge, and whether or not force production depends on engaging all the heads of a dynein arm. The choice of bridge force values in the model was based simply on matching the simulation output to the beating of real cilia and flagella. The close initial correspondence of the simulation-determined force values with published measurements is very encouraging.

If this proposed mechanism for flagellar oscillation is correct, several new concepts become central to understanding axonemal functioning. The first is the t-force itself, which appears to organize into patterns resembling solutions to Bessel’s equation. This may make possible more mathematically elegant descriptions of the t-force in the future.

A second major participant in the organization of the flagellar beat is side-to-side force transfer. The current model, as well as an earlier, less sophisticated version [Lindemann, 1994], both find that the optimal force transfer to reproduce cilia/flagella-like beating is between 0.1 and 0.3 (10–30%).

The role of the passive linkers is the third integral constituent in this view of how the axoneme functions. In order for the simulation to work at all, the links must be assigned an elastic constant ($K_E$). The value assigned to this constant affects the operation of the model. All sources appear to acknowledge the existence of interdoublet linkers. Several published measurements of the nexin link spacing generally agree on a repeat pattern of approximately 100 nm [Stephens, 1970; Warner, 1976; Witman et al., 1978; Burgess et al., 1991]. The high quality micrographs of Goodenough and Heuser [1985], and of Sugrue et al. [1991] also make a credible case for another set of peripheral links (in register with the 24 nm spacing of the dynein arms). These linkages were first mentioned by Witman et al. [1978], but have received less attention. Goodenough and Heuser’s work suggests these interdoublet links are extensions of the dynein stalks which remain attached to the B microtubule of the adjacent doublet. If this is so, these additional links would also have to be considered as part of the elastic component comprising $K_E$ in the model. There is also some controversy as to the persistence of the nexin connections throughout the beat cycle [Warner, 1983]. Consequently, a specific assignment of the elements responsible for the interdoublet elastic constant ($K_E$) is not possible without further experimental clarification. If we assume that only the nexin links contribute to the passive elastic force, then the value of $K_E$ can be used to assign an elastic constant to the individual nexin links (as was done in Table I). More likely, the relative contribution of two sets of links will complicate matters. Nonetheless, the total elastic constant of all linkages combined must still meet the same criteria.

Ciliary and flagellar arrest may have direct bearing on this issue. Both cilia and flagella will often arrest at one extreme of the beat cycle. If one assumes this is due to a failure in the switching mechanism, as Satir has suggested [Satir, 1985; Satir and Matsuoka, 1989], then the balance point observed in arrest amounts to a static balance between the forces of the active dynein bridges and the resistance of the passive linkages. In fact, if the computer simulation is allowed to arrest due to a switching failure, it assumes a final balance configuration resembling the switching arrest position of live cilia (Fig. 4C). The so-called “candy cane” shape observed in sea urchin sperm can also be generated in a longer simulation (30 μm) using the same procedure (Fig. 4D). If the assumption is correct that arrest represents a balance between the constraining passive elastic linkers and the bridge forces, then by choosing a $K_E$ value which gives a pattern of arrest matching that of real cilia and flagella, we can find reasonable values for $K_E$. The ciliary and flagellar simulations shown in Figures 6 and 7 were generated using $K_E$ values that produce life-like arrest patterns (shown in Fig. 4C and D).

It is well established that the motility of both cilia and flagella is regulated by cAMP dependent phosphorylation. At the same time, regulation of the dynein active site by phosphorylation has not yet been shown. The sites of phosphorylation that have been identified are on a regulatory light chain [Hamasaki et al., 1991], or on parts of the α heavy chain beyond the active site, and toward the base of the molecule [Stephens and Prior, 1992]. While these locations are not consistent with regulating the enzymatic site of the dynein, they could modulate arm flexibility or configuration, as was suggested by Stephens and Prior [1992]. In the context of this model, changes in the dynein stalk or the head configuration would directly affect the adhesion force mechanism (Fig. 3), and the base probability of bridge attachment. Both of these parameters are key values in establishing stable oscillations, and both can cause cessation of motion if adjusted out of the range that permits completion of an activity cycle.

In order to simulate a ciliary beat, it is necessary to use different initial or base probabilities ($Base.P$ in Equation 11) for bridge attachment of the P and R bridges. In real cilia this may correspond to either a very slight difference in the resting length of the passive links on the two sides of the axoneme, or a slightly modified dynein population on each side of the axoneme. Differences between the two sides of the axoneme, in sensitivity to
certain inhibitors, have been reported [Larsen and Satir, 1991; Lindemann et al., 1992; Kanous et al., 1993]. Small adjustments in the two initial probabilities can generate a wide range of symmetries in the resulting beat pattern [Lindemann, 1994]. In a living cilium or flagellum, the presence of a calcium-binding protein capable of changing shape or length in response to Ca\(^{2+}\) binding would be sufficient to change the base probability if it were located in the interdoublet links, or in the dynein regulatory complex on only one side of the axoneme. This could provide a mechanistic explanation for the diverse beating patterns observed in nature, with an elegant economy in the required modifications to the basic axonemal structure.

CONCLUSION

In conclusion, the proposed model provides an explanation for the process of dynein bridge switching based on the transverse forces generated within the beating axoneme. The hysteretic needed to permit each episode of bridge action to persist long enough to trigger bridge activation in the opposite bend direction is provided by adhesive forces contributed by attached dynein bridges. Bridge adhesion also provides a type of colligative excitability, making it possible for self-initiation of beating to occur. When this hypothetical picture of axonemal functioning is incorporated into a computer simulation, the simulation exhibits many properties of living cilia and flagella. The simulation can: (1) self-initiate motility, quickly stabilizing into a repetitive pattern of beating; (2) produce both cilia-like and flagellalike beating patterns, solely determined by the assigned length and relative probabilities of dynein bridge attachment in the two bend directions; and (3) function under the constraints of physical scale and measured properties of living cilia and flagella.

While this model of axonemal functioning is (at present) only a simplistic approximation, it supports the possibility that the switching mechanism described here may provide a key to understanding the beat cycle of living cilia and flagella.

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REFERENCES


